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Effect of ageing on the distribution and elimination of thiopentone in rats

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The effect of ageing on drug disposition in man has been described by Triggs & Nation (1975), Crooks et al (1976) and Vestal & Wood (1980), and in animals by Kato et al (1964), Kato & Takanaka (1968), Glants et al (1976), Kitani et al (1978), Klotz (1979) and Iga & Klaassen (1982). Age-related differences in drug disposition may be the result of progressive physiological changes as well as functional alterations in e.g. metabolism, excretion, tissue distribution and blood flow. Glants et al (1976) demonstrated a remarkable difference in the age-related pharmacokinetics of ouabain, a highly hydrophobic compound, between the young and older dogs, and Iga & Klaassen (1982) reported a similar age-related difference with ouabain between the young and older rats.

We set out to find the effect of ageing on the distribution and elimination of thiopentone by comparing the pharmacokinetic parameters obtained from rats at three different ages in an attempt to elucidate factors affecting age-related pharmacokinetics of thiopentone.

Methods

Adult Wistar (Nihon Seibutsu Zairyo, Tokyo, Japan) male rats were divided into the three groups: two-month old (2M) 268 ± 8 g, six-month old (6M) 619 ± 19 g, and twenty four-month old (24M) 595 ± 37 g. Under light ether anaesthesia, the femoral vein and artery were cannulated with polyethylene tubing (PE-50) for drug administration and blood sampling, respectively. After having recovered from anaesthesia, the rats were given 12 mg of thiopentone kg^{-1} (Tanabe Pharmaceutical Industry, Osaka, Japan) in 0·9% NaCl (saline) through the femoral vein cannula over 5 s with a 500 μl syringe. Blood samples (0·25 ml) then were obtained at 1, 5, 10, 30, 45, 60, 90, 120, 150 and 180 min in heparinized polyethylene centrifuge tubes (Beckman Instruments, Fullerton, Calif). The body temperature was kept at 37 °C by a heat lamp. Plasma was separated by centrifugation for 20 s and assayed for thiopentone, according to the method of Brodie et al (1950) as modified by Dayton et al (1967), in a Hitachi 356 dual beam spectrophotometer (λ_1 = 350 nm, λ_2 = 305 nm). The drug concentration data for individual animals were fitted to the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ for the plasma concentration C_t at time t by non-linear least squares regression (Nakagawa et al 1978). Pharmacokinetic constants were determined from the biexponential equation constants, i.e. A, α, B, and β, using conventional equations (Gibaldi & Perrier 1975). Volume of distribution at steady-state of unbound thiopentone, $Vd_{ss,f}$ was calculated by the equation of $Vd_{ss,f} = Vd_{ss}/f_p$ using each mean value of f_p, where f_p is the serum free fraction. The total body clearance (CL_{tot}) was calculated by the equation of CL_{tot} = dose/(A/α + B/β).

Serum was separated from the blood, obtained via the carotid artery, that had been standing for 60 min at room temperature (20°) by centrifugation for 10 min at 3000 rev min^{-1}. The f_p of thiopentone was determined by equilibrium dialysis at 37 °C for 16 h using semimicrocells (Kokugo-Gomu Co., Tokyo, Japan) and a semipermeable membrane (Type 36/32, Visking Co., Chicago, Ill) against 0·05 M isotonic tromethamine-hydrochloric acid buffer (pH 7·4), containing 0·038–0·113 mM thiopentone. The protein binding of thiopentone to serum was unchanged between 16 and 20 h of dialysis at 37 °C (Yu et al 1981). The serum albumin concentration was determined using a commercial kit (Daiichi Pure Chemical Co. Ltd, Tokyo, Japan).

Statistical analysis was performed using Student's *t*-test with $P = 0·05$ as the minimal level of significance.

Results and discussion

The plasma disappearance of thiopentone after intravenous administration of 12 mg kg^{-1} to 2, 6 and 24M rats is shown in Fig. 1. The disappearance of drug followed biexponential curves in all groups and no significant difference was observed among the three groups in the plasma concentrations or the pharmacokinetic constants β, Vd_{ss} and CL_{tot} (Table 1). The f_p of thiopentone

Table 1. Thiopentone pharmacokinetics in rats[a].

Constants	2 M[b]	6 M[b]	24 M[b]
A, μg ml^{-1}	14·8 ± 1·1	18·9 ± 1·5	27·0 ± 2·0
α, min^{-1}	0·296 ± 0·065	0·302 ± 0·018	0·201 ± 0·0450
B, μg ml^{-1}	18·2 ± 1·9	15·0 ± 1·0	14·6 ± 1·8
β, min^{-1}	0·0147 ± 0·0004	0·0148 ± 0·0024	0·0124 ± 0·0009
Vd_{ss}, ml kg^{-1}	719·6 ± 41·7	712·4 ± 44·8	669·1 ± 52·0
$Vd_{ss,f}$, ml kg^{-1}	4334·9 ± 251.2	3979·9 ± 250·3	2792·6 ± 217·0[c]
CL_{tot}, ml min^{-1} kg^{-1}	9·43 ± 0·95	10·93 ± 1·41	9·10 ± 0·58
f_p	0·166 ± 0·007	0·179 ± 0·006	0·240 ± 0·013[c]

[a] Results are given as the mean ± s.e.
[b] 2 M: 2 month old rats (n = 4), 6 M: 6 month old rats (n = 5), 24 M: 24 month old rats (n = 3).
[c] Significantly different from 2 M ($P < 0·05$).

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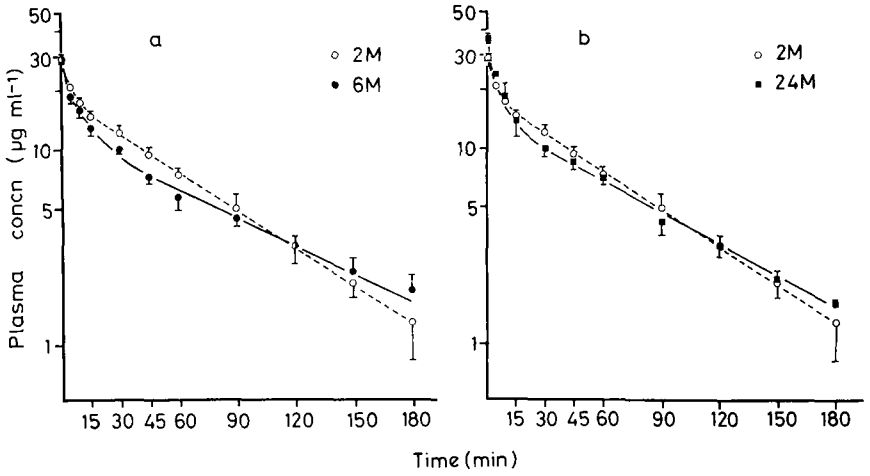

FIG. 1. Plasma disappearance curves of thiopentone after 12 mg kg^{-1} i.v. Each point and vertical bar represent the mean and standard error of three to five rats. Curves were calculated by the SALS method (Nakagawa et al 1978) using a digital computer. Panel a: (○) 2 month old rats (2M) and (●) 6 month old rats (6M); panel b: (○) 2M and (■) 24 month old rats (24M).

obtained from equilibrium dialysis is also shown in Table 1. No significant difference was observed in f_p between 2M and 6M rats, while in 24M rats there was a significant increase compared with that of 2M rats. The serum albumin concentrations of 2, 6 and 24M rats were 3·95 ± 0·23 (n = 3), 4·12 ± 0·11 (n = 3), and 1·90 ± 0·38 (n = 6) g dl^{-1}, respectively; no significant change was observed between 2M and 6M rats, while in 24M rats the concentration was decreased to approximately one-half that of 2M rats. A significant increase in f_p of the drug in 24M may be explained by the alteration of serum albumin concentration. The calculated $Vd_{ss,f}$ from Vd_{ss} and f_p are also listed in Table 1. No significant alteration was observed in 6M rats, while a significant decrease was observed in 24M rats, when compared with 2M rats. Thiopentone is bound in part to albumin in the tissue but mainly to lipids or other macromolecules, which have non-specific binding activities for thiopentone due to the high lipophilicity. It has been reported that much metabolically active tissue is replaced by fat; e.g. body fat increases from 18 to 36% of the total weight in man as age increases from 18 to 55 years (Novak 1972). Conceivably, such a change may result in the accumulation of highly lipophilic drugs in the tissue, but the results of our study were contrary to this consideration. The decrease in the tissue binding of thiopentone in 24M rats might be due to the changes in the contents of other macromolecules or the total body water.

In summary, the age-related changes in both the clearance and volume of distribution have been examined in rats. In 24M rats, a significant decrease was observed in the serum protein binding and also in $Vd_{ss,f}$, while no significant alteration was observed in CL_{tot}. These results may be explained by the decreased serum albumin concentration in the aged rats.

REFERENCES

Brodie, B. B., Mark, L. C., Papper, E. M., Lief, P. A., Bernstein, E., Rovenstine, E. A. (1950) J. Pharmacol. Exp. Ther. 98: 85–96.

Crooks, J., O'Malley, K., Stevenson, I. H. (1976) Clin. Pharmacokin. 1: 280–296

Dayton, P. G., Perel, J. M., Landrau, M. A., Brand, L., Mark, L. C. (1967) Biochem. Pharmacol. 16: 2321–2326

Gibaldi, M., Perrier, D. (1975) Pharmacokinetics, Dekker, New York, N.Y., pp 45–96

Glants, S. A., Kernoff, R., Goldman, R. H. (1976) Circ. Res. 29: 407–414

Iga, T., Klaassen, C. D. (1982) Proc. Soc. Exp. Biol. Med. 170: 59–62

Kato, R., Vassanelli, P., Frontino, G., Chiesara, E. (1964) Biochem. Pharmacol. 13: 1037–1051

Kato, R., Takanaka, A. (1968) J. Biochem. (Tokyo) 63: 406–408

Kitani, K., Kanai, S., Miura, R., Morita, Y., Kasahara, M. (1978) Exp. Geront. 13: 9–17

Klotz, U. (1979) Naunyn-Schmiedeberg's Arch Pharmacol. 307: 167–169

Nakagawa, T., Koyanagi, Y., Togawa, H. (1978) SALS, a Computer Program for Statistical Analysis with Least Squares Fitting, Library Program of the University of Tokyo Computer Center, Tokyo, Japan

Novak, L. P. (1972) J. Geront. 27: 438–493

Triggs, E. J., Nation, R. L. (1975) J. Pharmacokinet. Biopharm. 3: 387–418

Vestal, R. E., Wood, A. J. J. (1980) Clin. Pharmacokinet. 5: 309–319

Yu, H. Y., Sawada, Y., Sugiyama, Y., Iga, T., Hanano, M. (1981) J. Pharm. Sci. 70: 323–326